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<p>The conventional biochemical methods have not been very successful in providing the structural information for membrane proteins, including membrane-active peptides. Our methods, including oriented circular dichroism and x-ray scattering with the momentum transfer in the plane of the membrane, exploit the structural orders provided by the smectic liquid crystals composed of membranes. With these new methods, we have resolved some long-standing controversial questions about alamethicin and gramicidin, two extensively researched peptides. More importantly we discovered a new phase transition phenomenon of amphiphilic helical peptides: at low concentrations, the peptides are found to bind parallel to the membrane surface; above a lipid-specific critical concentration, the peptides transform into a different state, e.g., perpendicularly inserted in the membrane. These transitions appear to be related to the membrane selective cytolytic activity of these peptides. The latter have now been recognized as a new class of antibiotics.</p>					
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INTRODUCTION

The conventional biochemical methods have not been very successful in providing the structural information for membrane proteins, including membrane-active peptides. Our methods, including oriented circular dichroism and x-ray scattering with the momentum transfer in the plane of the membrane, exploit the structural orders provided by the smectic liquid crystals composed of membranes. With these new methods, we have resolved some long-standing controversial questions about alamethicin and gramicidin, two extensively researched peptides. More importantly we discovered a new phase transition phenomenon of amphiphilic helical peptides: at low concentrations, the peptides are found to bind parallel to the membrane surface; above a lipid-specific critical concentration, the peptides transform into a different state, e.g., perpendicularly inserted in the membrane. These transitions appear to be related to the membrane selective cytolytic activity of these peptides. The latter have now been recognized as a new class of antibiotics.

SPECIFIC ACCOMPLISHMENTS:

1. New Methods: In the past few years, we have opened a new line of research on membrane-active peptides with a new method of oriented circular dichroism and new x-ray techniques. We are now able to measure three structural parameters on a peptide interacting with a membrane, i.e., the orientation of the peptide, the lateral organization of the peptide in the plane of the membrane (the xy plane), and the z-coordinate of the peptide. In the following we describe these techniques and some results by these methods.

Method of oriented circular dichroism (OCD) for measuring peptide orientation: When a series of peptide bonds are arranged in a helix, quantum mechanics predicts that the peptide bond transition (e.g., $\pi\pi^*$) will be split into several components; and one of them (e.g., the band near 205 nm) is polarized along the helical axis (Moffitt, 1956; Olah and Huang, 1988a; 1988b). Making use of this special property, we invented a method of circular dichroism which can be used to determine the orientation of a helical peptide in a membrane (Wu, Huang and Olah, 1990).

OCD is the simplest method for determining the orientation of helices in membrane. It is the ease of this method that allowed us to examine peptides in many different chemical conditions and consequently led us to the discovery of the phenomenon of cooperative peptide transitions (Huang and Wu, 1991).

In-plane x-ray scattering for measuring peptide aggregation: The cooperative peptide transitions imply strong peptide-peptide interactions, most likely in some forms of aggregation. In order to measure such lateral organizations of peptides, we developed a technique of x-ray scattering with the momentum transfer confined in the plane of the membrane (He et al., 1992). The Fourier transform of the scattering intensity is then the correlation function of the peptide molecules in the plane.

Protein aggregations have been observed by freeze-fracture electron microscopy (e.g., Pearson et al., 1983). However the resolution of this technique is not fine enough to detect small peptides. Also the possibility of artifacts in the freeze-fracture process is difficult to assess (Pearson et al., 1984). Atomic force microscopy is potentially a powerful tool for imaging membrane proteins. But again the membrane has to be fixed, otherwise the probing force would move the molecules, making imaging impossible (Lacapere et al., 1992). Thus the greatest advantage of the scattering method is its applicability to the liquid crystalline (L_α) state of membranes.

As a demonstration of the in-plane scattering technique, we investigated the controversial question of whether gramicidin forms aggregates in a membrane. Our result showed that the scattering curve of the gramicidin is consistent with a random distribution of dimeric channels in the membrane. Thus we conclusively showed that gramicidin does not form aggregates (He et al., 1992).

Lamellar diffraction for measuring the z-coordinate of peptide: If a heavy atom is attached to a peptide (or if a hydrogen on the peptide is replaced by a deuteron), the z-coordinate of the label atom can be determined to within ± 0.3 Å by high-resolution x-ray (or neutron) lamellar diffraction. We demonstrated this technique by measuring for the first time the position of the ion-binding sites in the gramicidin channel (Olah et al., 1991; Liu et al., 1991; Huang et al., 1991).

The phase problem of lamellar diffraction is usually solved by the swelling method (Olah et al., 1991). However, this method does not always resolve the phases unambiguously. For this reason we developed an alternative method of phase determination by using anomalous dispersion (Liu et al., 1991; Huang et al., 1991).

2. New Phenomena: Magainins and many other 23 to 37 residue long peptides are newly discovered antimicrobial peptides that appear to be widely distributed in the animal kingdom as a universal means for defense against bacterial infections. What is unusual about these peptide antibiotics is that their target is the cellular lipid bilayer membrane. This is unlike other membrane-active proteins which exert their activity through specific receptors on the cell membrane. Without protein receptors, one might presume the peptide-membrane interactions are stoichiometric and nonspecific. But in fact there are critical concentrations for the peptide activity and these critical concentrations are membrane specific. Various magainin peptides are tumoricidal at concentrations 5-10 times greater than those required for antibiotic effects but 10-20 times less than those toxic to normally differentiated cells. We have discovered a new phenomenon of peptide-membrane interactions that can explain such self-catalytic, membrane-specific activity of the magainins. Many of these peptides, including magainins, form an amphipathic helical structure upon association with a membrane. We discovered that amphipathic helical peptides associate with a bilayer membrane in two ways; at low peptide-lipid molar ratios (P/L) the majority of peptide molecules adsorb on the membrane surface; but at high P/L the majority of peptide change into a different state; and most strikingly the majority's transition between the low-concentration state and the high-concentration (HC) state occurs over a narrow range of P/L, indicating that the peptide transition is a cooperative phenomenon; furthermore, the critical P/L value for the cooperative transition is different for different lipid compositions. We believe that the interaction of the HC state of the peptides with the cell membrane is the mechanism of cytotoxicity.

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